Increased Blood Flow Inhibits Neointimal Hyperplasia in Endothelialized Vascular Grafts


Intimal hyperplasia is a primary cause of failure after vascular reconstruction and may be affected by blood flow. We have studied the effects of increased blood flow on intimal hyperplasia in porous polytetrafluoroethylene grafts implanted in baboons. These grafts develop an endothelial lining by 2 weeks and neointimal thickening due to proliferation of underlying smooth muscle cells by 1 month. Creation of a distal arteriovenous fistula increased flow (from 230±35 to 785±101 ml/min, p<0.001) and mean shear (from 26±4 to 78±10 dynes/cm², p<0.001) without causing a drop in pressure across the grafts. Fistula flow did not alter the pattern of endothelial coverage but did cause a marked reduction in the cross-sectional area of the neointima (from 2.60±0.52 to 0.42±0.07 mm² at 3 months, p<0.01). Detailed morphometric analysis revealed an equivalent percentage decrease in smooth muscle cells and matrix content, suggesting that the primary effect of increased flow was to reduce smooth muscle cell number without affecting the amount of matrix produced by individual cells. The neointima remained sensitive to changes in flow at late times; ligation of the fistula after 2 months resulted in a rapid increase in neointimal thickness (from 0.60±0.03 mm² after 2 months of fistula flow to 3.88±0.55 mm² 1 month after ligation of fistula, p<0.01). These results support the hypothesis that changes in blood flow affect the structure of diseased as well as normal vessels. (Circulation Research 1991;69:1557–1565)

Intimal hyperplasia is the primary cause of restenosis in the first year after vascular reconstruction and is responsible for restenosis rates of up to 50% after percutaneous transluminal angioplasty. This process consists of smooth muscle cell (SMC) proliferation and matrix deposition and begins immediately after any type of arterial trauma. Once intimal thickening begins, there is no way to stop it. However, increased shear has been associated with reduced intimal thickening in early atherosclerotic plaques, experimental vein grafts, and injured arteries. These data suggest that maneuvers to increase blood flow across injured segments may reduce intimal hyperplasia.

It is not surprising that intimal thickening is influenced by shear, since arterial wall structure in both developing and mature vessels is modified by this hemodynamic force. Arterial diameter increases or decreases in response to changes in blood flow in a manner that maintains a constant shear force. Wall thickness, on the other hand, varies according to intraluminal pressure to maintain constant wall stress. The mechanisms for these adaptive processes are not known, but regulation of the function of SMCs must be involved, since these are the predominant cells in the vessel wall and they synthesize the extracellular matrix.

The effects of shear on vascular SMCs and endothelial cells have been studied in vitro, but these studies do not address the interaction of these two cell types or the possible effects of shear on mass transport at the interface of the flowing blood with the surface cells. In vivo study of the vessel wall response to altered blood flow is complicated by the fact that arteries change their diameter in response to changes in flow. These alterations in diameter cause both shear and wall tension to change; it then becomes difficult to determine the contribution of these two physical forces to any subsequent changes in wall structure.
The interplay of endothelium and underlying SMCs under varying conditions of blood flow in vivo might be studied independent of changes in vessel diameter in a system in which the vessel diameter is fixed and vasomotor function is abolished. We have developed such a system using highly porous polytetrafluoroethylene (PTFE) vascular grafts implanted into the arterial circulation of baboons. These grafts rapidly form a complete endothelial lining due to transmural ingrowth of microvessels. On reaching the lumen, the endothelial cells switch from a tubular morphology to a monolayer and spread. SMCs, presumably derived from pericytes, proliferate beneath this endothelium, thus producing a neointima that has the basic cellular components of the vessel wall. Unlike normal arteries, this structure is constrained by the rigid PTFE, which holds the external diameter constant. We have studied the effect of flow on neointima formation in this model by creating a small, non-pressure-reducing distal arteriovenous fistula.

Materials and Methods

Animal Model

Nonreinforced PTFE grafts (W.L. Gore and Associates, Inc., Flagstaff, Ariz.) with an intranodal distance of 60 μm, a length of 5–7 cm, and an internal diameter of 4 mm were implanted into the common iliac circulation of male baboons (Papio cynocephalus) 2 years of age weighing ~10 kg (Figure 1). Anesthesia was begun with an intramuscular injection of ketamine hydrochloride (10 mg/kg) and was continued with inhalational halothane. Grafts were preclotted with nonheparinized blood. The baboons were then heparinized, and the grafts were inserted between the infrarenal aorta and the common iliac artery using end-to-side Anastomoses created with 6-0 polypropylene suture. To prevent competitive flow through the native arterial circulation, the aorta was ligated below the proximal anastomoses, and the iliac arteries were ligated above the distal anastomoses. An incision was then made over the right groin vessels, and an 8–10-mm side-to-side anastomosis was made between the superficial femoral artery and vein using 7-0 polypropylene suture. This size fistula did not create a significant pressure reduction across the grafts (see below), and the distance between the fistulas and the grafts (~6 cm) was sufficient to prevent fistula-related flow disturbances in the grafts.

The effects of flow alteration on an established neointima were assessed in a reversal experiment in which the fistula was ligated after 2 months. At the same time, a similar fistula was created on the opposite (left) side. This second procedure was conducted under the same anesthetic as the first. These baboons were killed 1 month later (3 months after graft placement), and their neointimas were compared with those from grafts that had 3 months of uninterrupted control or fistula flow.

Animal care complied with the “Principles of Laboratory Animal Care” (formulated by the National Society for Medical Research) and the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health publication No. [NIH] 80–23, revised 1985).

Hemodynamic Measurements

Velocity and pressure measurements were made immediately before death in anesthetized baboons. The abdomen was opened, and the aorta and iliac arteries were exposed. Blood pressures were measured with a strain gauge and direct needle puncture of the aorta just proximal to the grafts and of the iliac arteries just distal to the grafts. Center stream blood velocities were measured using a 20-MHz pulsed Doppler probe (Hartley, Houston, Tex.), which was placed directly on the vessels at a 60° angle. The Doppler-shifted signal was processed with a spectrum analyzer (MedaSonic, Mountain View, Calif.), and hard copies of the velocity waveforms were obtained using a thermal printer. Frequency shifts were converted to velocity using the Doppler equation. Peak velocities were measured from the hard copy, and time-averaged velocities were obtained by planimetry.

In some baboons, velocities were measured at various times using a duplex scanner (Acuson, Mountain View, Calif.). This technique allowed measurements to be made through the intact abdominal wall with the baboon under light ketamine sedation. Peak and time-averaged velocities were calculated through software in the duplex scanner.

Wall shear stress (τ) in dynes per square centimeter was calculated using the Hagen-Poiseuille formula

$$τ = 4\mu Q / π r^3$$
where Q is volume flow, μ is the kinematic viscosity of blood (assumed to be 0.035 poise), and r is the vessel radius. Volume flow was calculated as the product of the measured graft luminal area and the time-averaged velocity. Flow calculations made with this method agreed well with simultaneous timed blood collection at the time of death (n = 10, data not shown).

**Morphology**

The baboons were killed at 2 weeks and 1, 2, and 3 months after graft placement. One-half hour before death, anesthetized baboons were given intravenous injections of heparin (3,000 units) and Evans blue dye (50 mg/kg body wt, 50 mg/ml in normal saline).

The baboons were killed by anesthetic overdose and exsanguination. They were perfused with Ringer’s solution via an axillary artery cannula at 100 mm Hg for 15 minutes and were then perfusion-fixed with 2.5% glutaraldehyde in 0.1 M phosphate-buffered saline, pH 7.4. Venous drainage was provided through incisions in the femoral veins. The aorta, iliac arteries, femoral vessels at the level of the fistula, and the grafts were removed en bloc and immersed in the same fixative for at least 24 hours. Specimens were then trimmed of excess tissue and washed in multiple changes of buffer followed by 0.1 M glycine buffer, pH 7.4.

Grafts were cut into 0.5-cm rings. Because plastic does not permeate PTFE, materials were embedded in paraffin with care being taken to preserve the proper tissue orientation. Cross sections were stained with hematoxylin and eosin. Area measurements were performed with computer-assisted morphometry using a digitizing pad and camera lucida on a standard light microscope. By using the highest magnification that allowed the entire graft section to be visualized in one field, the area of the neointima was established by tracing first its inner border (the junction of the endothelial lining with the lumen) and then its outer border (the junction of the graft with the neointima). Data analysis used measurements averaged from three or four cross sections from the middle of each graft; anastomoses were excluded.

Sections from the midportion of the grafts were processed for scanning electron microscopy. These were dehydrated through increasing concentrations of ethanol and dried with liquid carbon dioxide in a critical point dryer. Specimens were then placed on stubs and sputter-coated with gold palladium for viewing with a scanning electron microscope (model 35 C, JEOL U.S.A., Inc., Peabody, Mass.).

**Autoradiography**

Autoradiography was performed at the 1- and 3-month time intervals. (It is not possible to determine thymidine labeling indexes at earlier times because there are too few SMCs in the neointima.) Tritiated thymidine (6.7 Ci/mM, 0.5 mCi/kg body weight, New England Nuclear, Inc., Boston) was given by intramuscular injection at 1, 9, and 17 hours before death. All cells proliferating in a 24-hour period should be labeled by this technique, since the DNA synthetic phase is ~8 hours.39 Unstained, deparaffinized 6-μm histological cross sections on glass slides were dipped in Kodak NTB-2 emulsion, stored at 4°C for 2 weeks, developed with Kodak D 19 developer, and stained with hematoxylin. Labeled nuclei were identified under oil immersion light microscopy by the presence of silver grains (five grains or more). The total number of nuclei in each area was calculated by multiplying the neointimal cross-sectional area by the number of nuclei per square millimeter (estimated by counting nuclei in eight representative defined areas using a light microscope with a reticle). The thymidine labeling index (percent) was calculated as 100 times the number of labeled nuclei divided by the total number of nuclei in the section.

**Estimation of Smooth Muscle Cell Mass**

The area of the neointima may be altered by changes in the SMC content or changes in the amount of matrix they produce. To assess the relative contribution of these two components to neointimal thickening, we determined the percentage of the neointimal area that was occupied by SMCs. This was measured from transmission electron photomicrographs using the point-hit method.30 This analysis was performed on grafts from two baboons killed at 2 months and from two baboons from the reversal experiment killed at 3 months. Neointimas were separated from grafts that had been embedded in paraffin and were reembedded in Epon for preparation of thin sections for transmission electron microscopy. Tissue for sectioning was taken from as many locations around the perimeter of the neointima as possible. Because thin neointimas were particularly difficult to embed, only four to eight different areas could be processed from each graft, whereas thicker neointimas yielded as many as 10 separate locations for analysis. Photographs (8×10 in.) of specimens from each embedded block were obtained in a manner that covered the entire width of the specimen from the inner to outer surface. These were taken at a magnification of ×3,000 and printed at a final magnification of ×9,000. A 9×12 grid (108 points, 2 cm apart) was placed over the photograph, and the number of points overlaying SMCs were counted. This yielded from 2,302 to 9,717 test points for each neointima. Given this number of points and the fact that SMCs occupy ~30% of the volume, the expected relative error in estimating volume density is ±3%.30 Since the neointima essentially is composed only of SMCs and matrix, the percentage of neointimal area occupied by matrix was calculated as 100 minus the percentage occupied by SMCs.

**Statistics**

Comparisons among means of neointimal areas were made using analyses of variance, adjusting for multiple comparisons by the Scheffe’s method (BMDP Program 7D, BMDP Statistical Software Inc., Los
Table 1. Hemodynamic Measurements in Baboons

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fistula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure drop across graft (mm Hg)</td>
<td>4.6±1.5</td>
<td>6.0±1.7</td>
</tr>
<tr>
<td>Flow (ml/min)</td>
<td>230±35</td>
<td>785±101*</td>
</tr>
<tr>
<td>Peak systolic velocity (cm/sec)</td>
<td>128±13</td>
<td>217±26*</td>
</tr>
<tr>
<td>Time-averaged velocity (cm/sec)</td>
<td>35±5</td>
<td>109±14*</td>
</tr>
<tr>
<td>Average shear (dynes/cm²)</td>
<td>26±4</td>
<td>78±10*</td>
</tr>
</tbody>
</table>

Pressures are mean±SEM of measurements made when the baboons were killed: at 2 weeks (n=2), 1 month (n=3), and 2 months (n=5). All other variables are mean±SEM of single measurements from 16 baboons. Measurements were made at 1 week (n=3), 2 weeks (n=2), 1 month (n=4), 2 months (n=2), and 3 months (n=5).

*p<0.001 compared with control by paired t test.

Results

Hemodynamics

The mean decrease in peak systolic pressure across the grafts was 5 mm Hg and was not significantly different on the control and fistula sides (Table 1). Calculated blood flow on the side of the fistula was significantly higher than flow on the control side (Table 1).

The velocity waveform was altered by the fistula (Figure 2). While the control side had no forward flow during diastole and sometimes a brief period of reversed flow, the fistula side had significant forward flow throughout the cardiac cycle. Flow on the control side was fairly uniform, but disturbed flow (spectral broadening) was evident on the side of the fistula. Both peak systolic velocity and time-averaged velocity were significantly increased on the fistula side (Table 1). Calculated average shear stress was also significantly higher in grafts with fistula flow (Table 1).

In reversal experiments, velocity waveforms on the left side, which had a fistula created, changed from the typical control pattern to one of fistula flow with peak velocities of ~175 cm/sec and significant forward flow throughout diastole. On the right side, where the fistula was closed, flow patterns changed from those typical of fistula flow to those typical of control flow with a peak systolic velocity of ~110 cm/sec and flow to zero in diastole.

Morphology

At 2 weeks, the surface of all grafts was completely covered by cells that had the appearance of endothelium on scanning electron microscopy (n=2 baboons). Capillary orifices were scattered throughout the luminal surface (Figure 3). In previous studies we have shown that these surface cells stain for factor VIII-related antigen.28

In general, neointimal thickness was uniform throughout the length of each graft; there was no increase at the anastomoses. Neointimal cross-sectional area was decreased on the side of the fistula at all times studied (Table 2, Figures 4 and 5). These differences were statistically significant at 2 and 3 months (p<0.01).

In reversal experiments, fistula closure caused increased neointimal thickening; at 3 months, grafts on the right side, where the fistula was closed after 2 months, had significantly more neointima than grafts that had 3 months of uninterrupted fistula flow (p<0.01, Table 2, Figure 5). Grafts on the left, which had fistula flow created at 2 months, had slightly less neointimal area than control grafts examined at 3 months, but this difference was not statistically significant.

The percentage of the neointima that was composed of SMCs was similar in specimens with control and fistula flow (Table 3). The percentage of the neointimal area occupied by SMCs varied within the neointima and was significantly higher in the area immediately beneath the endothelium than in all other regions of the neointima (39.7±13.5% under-
neath the endothelium versus 24.9±11.7% elsewhere, \( p<0.001 \).

*** Autoradiography ***

SMC thymidine labeling indexes were the same on
the control and fistula sides at both 1 month
(1.5±1.3% versus 1.8±1.7%, \( n=4 \)) and 3 months
(0.03±0.01% versus 0.04±0.04%, \( n=4 \)). Rates of
proliferation at 3 months were at quiescent levels.

** Discussion **

The neointima of porous PTFE grafts in baboons is
composed of vascular endothelium, underlying
SMCs, and matrix produced by these cells. Therefore,
this system has the basic components of the
intima of injured or atherosclerotic vessels and provides
a model for studying the effects of shear on new
intimal growth. Because PTFE is rigid, shear can be
varied without affecting other parameters, such as
vessel diameter and wall stress. Our finding that
increased blood flow results in decreased neointimal
area suggests a mechanism for control of SMC
growth in response to changes in shear. It is not
surprising that such a mechanism exists, since shear
rate is known to affect arterial structure in both
normal and diseased vessels.

During development, arterial caliber varies with
flow and therefore with shear rate, which is directly
proportional to blood flow.18–21 The diameter of
mature arteries also changes in response to altered
flow in a manner that maintains shear at a constant
level. Several investigators have noted such arterial
dilatation in response to increased flow caused by
creation of an arteriovenous fistula22–25; we also
observed dilatation of the iliac arteries supplying the
fistula in our experiment (data not presented). In
contrast, reduced flow results in reduction of arterial
diameter.26 Langille and O'Donnell26 observed a

** Figure 3. ** Scanning electron micrograph of the surface of a polytetrafluoroethylene vascular graft with fistula flow at 2 weeks.
Note that the endothelial lining is complete. A capillary orifice is seen at the top. Bar, 100 \( \mu \)m.

** Table 2. ** Cross-sectional Neointimal Areas in Baboons

<table>
<thead>
<tr>
<th>n</th>
<th>Normal flow (left)</th>
<th>Fistula flow (right)</th>
<th>Cross-sectional neointimal area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Uninterrupted experiments</strong></td>
</tr>
<tr>
<td>1 mo</td>
<td>4  1.81±0.44</td>
<td>0.79±0.35*</td>
<td>n</td>
</tr>
<tr>
<td>2 mo</td>
<td>3  4.39±0.03</td>
<td>0.60±0.03†</td>
<td>.</td>
</tr>
<tr>
<td>3 mo</td>
<td>7  2.60±0.52</td>
<td>0.42±0.07†</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. \( n \). Number of grafts. The 3-month value for the fistula created at 2 months represents 2 months of normal flow
followed by 1 month of fistula flow; the 3-month value for the fistula closed at 2 months represents 2 months of fistula flow followed by 1
month of normal flow.

*\( p=NS \) and \( tp<0.01 \) vs. corresponding value for normal flow (left) by paired \( t \) test; \( tp=NS \) vs. corresponding value for normal flow (left)
by unpaired \( t \) test; \( tp<0.01 \) vs. corresponding value for fistula flow (right) by unpaired \( t \) test.
21% decrease in carotid artery diameter in the rabbit when flow was reduced by 70%. This diameter reduction was not reversed by smooth muscle relaxants, implying that altered flow resulted in a structural modification of the wall rather than a change in SMC tone.

Shear also modulates wall structure in diseased vessels. Human atherosclerotic plaques and early lesions in fat-fed animals are more prone to develop in areas of low shear. Similarly, wall thickening of vein grafts placed in the arterial circulation is greatest in areas of decreased flow. Finally, studies by Glagov et al. suggest that human coronary arteries dilate when atherosclerotic plaque narrows the lumen; this compensatory mechanism helps maintain normal shear and luminal area in the presence of mild to moderate encroachment of the lumen by plaque. Neointimal thickening in our rigid PTFE model may most closely mimic lesion progression in these rigid, calcified atherosclerotic arteries. It seems likely that important features of the regulation of wall structure in response to alterations in shear are common to graft neointima, early atherosclerosis, vein graft thickening, and native vessels, since all have the same basic structure consisting of an endothelial surface lining with underlying SMCs and the matrix they produce.

In our experiments, decreased graft neointimal thickening on the side of the fistula appeared to be due to reduction in both SMC and matrix content, since the volume fractions of SMCs and matrix did not change. This finding is explained by a reduction in SMC number without a change in the amount of matrix each cell produces. We would thus expect SMC proliferation to be less in the fistula group, yet thymidine labeling indexes were not different at 1 or 3 months. These are relatively late times, and it is likely that most of the SMC proliferation takes place.

Table 3. Composition of Neointima in Baboons

<table>
<thead>
<tr>
<th>Baboon</th>
<th>Control</th>
<th>Fistula</th>
<th>Fistula closed at 2 mo</th>
<th>Fistula opened at 2 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.8</td>
<td>28.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24.4</td>
<td>33.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>...</td>
<td>32.3</td>
<td>27.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>...</td>
<td>...</td>
<td>27.8</td>
<td>28.1</td>
</tr>
</tbody>
</table>

Values are means for four individual baboons.

Figure 4. Cross sections of grafts removed at 3 months. Panel A: Control flow. Panel B: Fistula flow. The arrows indicate the junction of the graft and neointima. Bar, 100 μm.

Figure 5. Graph showing neointimal cross-sectional areas for control flow (left side, open circles), fistula flow (right side, open squares), and for the reversal experiments at 3 months (left side, fistula created at 2 months, closed circle; right side, fistula closed at 2 months, closed square). Values indicated are mean±SEM. See Table 2 for the number of graft pairs at each time point and significance levels.
during the first few weeks, as it does when neointimal forms after arterial injury.29

We calculated mean shear stress using the Hagen-Poiseuille formula, because it is not possible to directly measure shear stress at the wall. This method gives only a rough approximation of actual shear levels and depends on many assumptions, including absence of turbulence. Turbulence generally occurs at Reynolds numbers above 2,000 (the Reynolds number equals UD/μ, where U is the velocity, D is the vessel diameter, and μ is the kinematic viscosity of blood). Time-averaged velocities used to calculate shear stress in our model resulted in calculated Reynolds numbers that were well below 1,000. Calculated shear stress in the grafts with fistula flow was two to three times that of the control grafts (78±10 versus 26±4 dynes/cm²) and was outside the physiologic range for arteries, which is 10–20 dynes/cm².25 In addition, grafts on the side of the fistula had significant forward flow throughout the cardiac cycle, while flow on the control side usually went to zero or even reversed in diastole. Thus, the fistula changed both the magnitude of the shear and the amount that it varied over time. The observed difference in development of graft neointima may have been caused by either or both of these changes.

We have attempted to determine whether neointima responds to the magnitude of shear or to the amount that it varies over time using a model in which animals with bilateral aortoiliac PTFE grafts also had a more proximal PTFE graft placed.32 These proximal grafts supplied both of the two iliac grafts and thus carried twice as much blood flow and had twice the average shear. Nevertheless, their velocity waveforms were similar in shape to those of the more distal grafts, including the presence of flow to zero in diastole. Proximal grafts had significantly less neointimal area than the distal ones, suggesting that the absolute value of the shear force rather than the magnitude of its variation over time is the important determinant of neointimal growth. The magnitude of shear in these proximal grafts was much nearer to physiological levels than it was in grafts with fistula flow, suggesting that this mechanism of neointimal growth regulation may be important at clinically relevant flow rates.

The endothelium probably plays a regulatory role in this adaptation of wall structure to altered shear, since it, rather than the SMCs beneath, is in contact with the flowing blood. Langille and O’Donnell26 have pointed out that it is unlikely that the SMCs within the wall can directly sense changes in shear stress, since even high shear rates (like those produced by the fistula in our experiments) cause <1% shear strain (deformation of the wall). Therefore, in order for shear to affect wall structure, a signal must be generated from the lining cells, which are in contact with the flowing blood, to the SMCs beneath. There is evidence that an intact endothelium is indeed necessary for the arterial response to both increased and decreased blood flow.23,26

Many endothelial functions are affected by flow, including endocytosis, proliferation, cell shape, cytoskeletal organization, and potassium flux.33–36 In vitro studies suggest that surface reactive forces may affect intracellular signaling pathways in endothelial cells, including activation of potassium current37 and increasing inositol triphosphate levels.38 Shear could influence SMC proliferation by modulating endothelial cell production of SMC regulatory molecules. The endothelium is capable of producing many such factors, including both growth promoters (transforming growth factor β, platelet-derived growth factor [PDGF], fibroblast growth factor [FGF], interleukin-1, and epidermal growth factor) and growth inhibitors (heparin sulfate and endothelium-derived relaxing factor [EDRF]).39–42 Our results could be explained either by increased production of growth inhibitors or by reduced production of growth promoters under conditions of increased flow. Endothelial production of at least one growth inhibitor, EDRF, is known to be influenced by flow. Nitric oxide-generating vasodilators and 8-bromo guanosine 5’-monophosphate can inhibit SMC proliferation in vitro, suggesting that EDRF may function as a modulator of SMC proliferation through a cGMP-mediated pathway.43 Several studies have shown that production of EDRF is influenced by flow in vitro,40,41 but it is not known if this mechanism is active in vivo.

PDGF production by endothelial cells is also influenced by flow in vitro,42 and previous work from our laboratory suggests that this mitogen is an important regulator of SMC proliferation in PTFE grafts.44 Perfsusates from excised grafts have mitogenic activity that is inhibited by PDGF-blocking antibodies. In situ hybridization techniques have localized PDGF-A chain to the endothelium and to a lesser degree to the underlying SMCs of the neointima, and PDGF mRNA has been detected in neointima and in SMCs cultured from PTFE grafts.45 Furthermore, thymidine autoradiographic studies have demonstrated that most of the SMC proliferation occurs in the region just underneath the endothelium, where PDGF is also found.45 Basic FGF may also be important in healing graft neointima. In preliminary studies, we have demonstrated patchy staining for basic FGF in the endothelium and have found the same amount of basic FGF mRNA in the graft neointima as in the adjacent aorta (unpublished data). Decreased neointimal area in grafts with high flow could be explained by reduced production of mitogens such as PDGF or basic FGF.

Matrix synthesis may also be affected by growth regulatory factors. Detailed morphometric analysis of local SMC and matrix content in our model suggests that the inner region of the neointima, where most proliferating SMCs are located, also contains significantly less matrix than the remainder of the neointima.
Our reversal experiment demonstrated that mature neointima remains sensitive to flow. Ligation of the fistula after 2 months was followed by an increase in neointimal area. This increase is likely due to proliferation and matrix production by SMCs in the neointima, perhaps in response to increased production of growth factors. As noted above, basic fibroblast growth factor and PDGF are expressed in the neointima in regions containing proliferating SMCs. If these growth factors have an important role in neointimal SMC proliferation, we would expect them to be increased by the switch from high to normal flow. Furthermore, if our earlier observations are correct, the increase in growth factor expression might be located in the inner neointima and dependent on the presence of endothelium.

In summary, we have presented evidence that formation of neointima in PTFE grafts is reduced when flow is increased by creation of a distal arteriovenous fistula. Altered flow appears to affect both SMC and matrix content. Relatively mature neointima is responsive to flow alteration at late times; it can increase its mass when flow is reduced and may be able to decrease its mass when flow is augmented. These effects are likely to be mediated by the endothelium through differential production of mitogens or growth inhibitors. Because the neointima is structurally similar to intima in vein grafts and early atherosclerotic plaques, greater understanding of the regulation of this proliferative response may yield insight into mechanisms that control intimal hyperplasia and into the development of atherosclerotic lesions.

Acknowledgments

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References


Key Words • intimal hyperplasia • blood vessel prosthesis • vascular smooth muscle • blood flow velocity • Papio arteriovenous fistula • vascular graft occlusion
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